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L3: Entry 11 of 41 File: PGPB Oct 24, 2002

DOCUMENT-IDENTIFIER: US 20020155099 A1
TITLE: TRANSFER OF MOLECULES INTO THE CYTOSOL OF CELLS

Detail Description Paragraph (75):
[0096] An ODN-DOTAP (DOTAP is an abbreviation for (N-[1-(2,3-dioleoyloxy) propyl]-N,N,N-trimetylammonium methyl sulphate) complex was formed by gently mixing 5 .mu.g ODN (same as in Example 14) in 42 .mu.l 20 mM Hepes buffer (HBS) pH 7.4 with 25 .mu.g DOTAP (Boehringer Mannheim) in 84 .mu.l HBS (25 .mu.g DOTAP per 5 .mu.g ODN and the volume of ODN mixture is half of the DOTAP mixture volume). After 15 min incubation at room temperature the solution was diluted with culture medium to a final volume of 1 ml and added to the cells (1 ml per well). 100 000 THX-cells were seeded out into a Falcon 3001 dish. After overnight incubation 20 .mu.g/ml AlPcS.sub.2a was added and the cells were incubated for 18 h at 37.degree. C. The cells were washed 3 times with culture medium and incubated in sensitizer-free medium for additional 3 h before being transfected with the ODN-DOTAP complex for 4 h. After transfection the cells were incubated in culture medium for 2 h and unfixed cells were observed for the intracellular localization of ODN and AlPcS.sub.2a with fluorescence and phase-contrast microscopy (objective with 40 times magnification, the microscope was equipped with a 450-490 nm band pass excitation filter). For relocalization studies the cells were exposed to microscope light for 10 s, and after 1 min (relocalization time) fluorescence pictures were taken.

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L3: Entry 13 of 41 File: PGPB Sep 5, 2002

DOCUMENT-IDENTIFIER: US 20020122819 A1
TITLE: Novel liposome complexes for increased systemic delivery

Brief Description of Drawings Paragraph (10):
[0028] FIG. 4. Proposed model showing cross-sections of DOTAP:Chol liposomes interacting with supercoiled plasmid DNA. The X indicates fusion of lipid bilayers. The enlarged area shows proposed arrangement of DNA condensed between two 4 nm bilayers of DOTAP:Chol.

Detail Description Paragraph (88):
[0101] When these liposomes were complexed with DNA at optimal concentrations, with a .+-. charge ratio .rho.=2 (Lasic et al. (1997)), the DNA was localized to the interior of the liposomes (FIG. 3B). DOTAP:DOPE (dioleoyl phosphatidyl-ethanolamine) and DOTAP:Chol complexes with DNA were turbid colloidal solutions with mean particle size of 445 nm and 405 nm, respectively. Particle size did not depend on dilution, and turbidity obeyed Beer Lambert Law indicating stability of these complexes in vitro. DOTAP:DOPE liposomes also form "vase structures"; however, the orifices were larger and many spheres were formed (FIG. 3E). In addition, there were many structures with little or no DNA assembled in the extruded DOTAP:DOPE liposomes (FIG. 3F), and the DNA was frequently found on the outside of these liposomes (FIG. 3F). DDAB:DOPE and DDAB:Chol liposomes did not form "vase structures". The internalization of DNA within "vases" is a unique feature of extruded DOTAP liposomes and have not been observed for any other DNA:liposome complex studied by cryo-electron microscopy (Frederik et al. (1991)). The "vase structures" observed for DOTAP:Chol may contribute to the high systemic delivery and gene expression achieved with these formulations.

L3: Entry 22 of 41 File: USPT Oct 22, 2002

DOCUMENT-IDENTIFIER: US 6468798 B1
TITLE: Expression of cloned genes in the lung by aerosol and liposome-based delivery

Detailed Description Text (94):
Liposomes were prepared as small unilamellar vesicles (approximately 100 nm in diameter) containing the cationic lipid DDAB (dimethyl dioctadecyl ammonium bromide) as DDAB cholesterol in a 1:1 molar ratio. DDAB was purchased from Sigma, St. Louis, Mo., and cholesterol was purchased from CalBioChem, San Diego, Calif. Stock solutions of the lipids were dissolved in chloroform. Lipids were mixed in a round-bottomed flask and evaporated to dryness on a rotary evaporator under reduced pressure. Double distilled water was added to produce final lipid concentrations of 10 mM each, and the resulting mix was sonicated for approximately 20 minutes in a bath sonicator (Laboratory Supplies, Hicksville, N.Y.).

L3: Entry 24 of 41 File: USPT Jul 2, 2002

DOCUMENT-IDENTIFIER: US 6413544 B1
TITLE: Liposome complexes for increased systemic delivery

Drawing Description Text (10):
FIG. 4. Proposed model showing cross-sections of DOTAP:Chol liposomes interacting with supercoiled plasmid DNA. The X indicates fusion of lipid bilayers. The enlarged area shows proposed arrangement of DNA condensed between two 4 nm bilayers of DOTAP:Chol.

Detailed Description Text (88):
When these liposomes were complexed with DNA at optimal concentrations, with a+/- charge ratio .rho.=2 (Lasic et al. (1997)), the DNA was localized to the interior of the liposomes (FIG. 3B). DOTAP:DOPE (dioleoyl phosphatidyl-ethanolamine) and DOTAP:Chol complexes with DNA were turbid colloidal solutions with mean particle size of 445 nm and 405 nm, respectively. Particle size did not depend on dilution, and turbidity obeyed Beer Lambert Law indicating stability of these complexes in vitro. DOTAP:DOPE liposomes also form "vase structures"; however, the orifices were larger and many spheres were formed (FIG. 3E). In addition, there were many structures with little or no DNA assembled in the extruded DOTAP:DOPE liposomes (FIG. 3F), and the DNA was frequently found on the outside of these liposomes (FIG. 3F). DDAB:DOPE and DDAB:Chol liposomes did not form "vase structures". The internalization of DNA within "vases" is a unique feature of extruded DOTAP liposomes and have not been observed for any other DNA:liposome complex studied by cryo-electron microscopy (Frederik et al. (1991)). The "vase structures" observed for DOTAP:Chol may contribute to the high systemic delivery and gene expression achieved with these formulations.

L3: Entry 32 of 41 File: USPT Nov 7, 2000

DOCUMENT-IDENTIFIER: US 6143716 A
TITLE: Liposomal peptide-lipid conjugates and delivery using same

Detailed Description Text (56):
Collected lipid was dried under N.sub.2 stream and exposed to vacuum for 4 hours-overnight. Samples were resuspended in 100% ethanol and injected in 30 ul aliquots into Spherisorb silica columns (150.times.4.6 mm, 0.3 um, Keystone Scientific). HPLC was performed using a hexane:isopropanol:water:TFA mobile phase. Hexane and TFA were held constant at 37% and 0.2%, respectively. The N-Ac-AA-DOPE peak was detected using a gradient of 59-55% isopropanol:4-8% water. Flow rate was 1.5 ml/min, column temperature was set at 45.degree. C., and peaks were detected by a UV detector set at 205 nm. Lipid peaks were quantitated in comparison to standard curves generated by injecting 5-200 nmol of DOTAP or N-Ac-AA-DOPE and monitoring 205 nm signal. % cleavage was calculated by normalizing peaks to DOTAP, then determining the decrease in N-Ac-AA-DOPE peak size relative to starting amounts.

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L3: Entry 39 of 41 File: USPT Aug 26, 1997

DOCUMENT-IDENTIFIER: US 5660855 A
TITLE: Lipid constructs for targeting to vascular smooth muscle tissue

Detailed Description Text (24):
Typically, liposomes were sonicated until greater than 85% of the liposomes were less than 300 nm diameter. This allowed sterile filtration using a 450 nm cellulose acetate filter without significant liposome loss on the filter. All liposome formulations except DOTAP:DOPE:Amch (1:1:1), DODAP:DOPE:Amch (1:1:1) and DOSPA:DOPE:Amch were 10-100 nm average diameter. DOTAP:DOPE:Amch (1:1:1) and DODAP:DOPE:Amch(1:1:1) averaged 100-200 nm diameter; DOSPA:DOPE:Amch were greater than 1 .mu.m and could not be sterile filtered.

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